Journal of Medical Virology 57:186–192 (1999)

Respiratory Syncytial Virus Infection Prolongs Methacholine-Induced Airway Hyperresponsiveness in Ovalbumin-Sensitized Mice

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Severe respiratory syncytial virus (RSV)-induced disease is associated with childhood asthma and atopy. We combined models of allergen sensitization and RSV infection to begin exploring the immunologic interactions between allergic and virus-induced airway inflammation and its impact on airway hypersensitivity. Airway resistance was measured after methacholine challenge in tracheally intubated mice by whole body plethysmography. Lung inflammation was assessed by bronchoalveolar lavage (BAL) and histopathology. RSV infection alone did not cause significant airway hyperresponsiveness (AHR) to methacholine. Ovalbumin (OVA)-induced AHR lasted only a few days past the discontinuance of OVA aerosol in mice that were ovalbumin sensitized and mock infected. In contrast, OVA-sensitized mice infected with RSV during the OVA aerosol treatments (OVA/RSV) had AHR for more than 2 weeks after infection. However, 2 weeks after either RSV or mock infection, OVA/RSV mice had significantly more lymphocytes found during BAL than OVA mice, whereas the OVA and OVA/RSV groups had the same number of eosinophils. Histopathologic analysis confirmed an increased inflammation in the lungs of OVA/RSV mice compared with OVA mice. In addition, OVA/RSV mice had a more widespread distribution of mucus in their airways with increased amounts of intraluminal mucus pools compared with the other groups. Thus, prolonged AHR in RSV-infected mice during ovalbumin-sensitization correlates with increased numbers of lymphocytes in BAL fluid, increased lung inflammation, and mucus deposition in the airways, but not with airway eosinophilia. A further understanding of the immunologic consequences of combined allergic and virus-induced airway inflammation will impact the management of diseases associated with airway hyperreactivity. ***J. Med. Virol. 57:186–192, 1999.***

KEY WORDS: RSV; ovalbumin; mice; asthma; lymphocytes

INTRODUCTION

Viral infections and atopy are strongly associated with the development of reactive airway disease. Between 80–85% of asthma exacerbations in school-age children are associated with viral respiratory tract infections [Johnston et al., 1995], and there is a significant relationship between viral infections and severe attacks of asthma that lead to hospital admission [Johnston et al., 1996]. Immediate hypersensitivity to aeroallergens is considered to be the strongest predictor of asthma [Anonymous, 1995]. Approximately 90% of individuals with asthma under the age of 30 are atopic [Smith, 1974]. The combined effect of viral infection and allergic sensitivity leads to increased allergen-induced airway responsiveness [Lemanske et al., 1989].

Respiratory syncytial virus (RSV) is the most common viral cause of severe lower respiratory tract disease in childhood [Glezen and Denny, 1973], usually manifested as a bronchiolitis with wheezing [McIntosh et al., 1973]. In a small minority of children, RSV infection leads to severe disease requiring hospitalization. Severe RSV-induced disease is associated with childhood asthma and repeated episodes of bronchospastic bronchitis, which can continue into adulthood [Sigurs et al., 1995]. There is also evidence that severe RSV-induced disease may be associated with a predis-

Grant sponsor: NIH; Grant numbers: RO1-AI-33933, GM 15431, K08-HL-03730.

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Accepted 24 August 1998

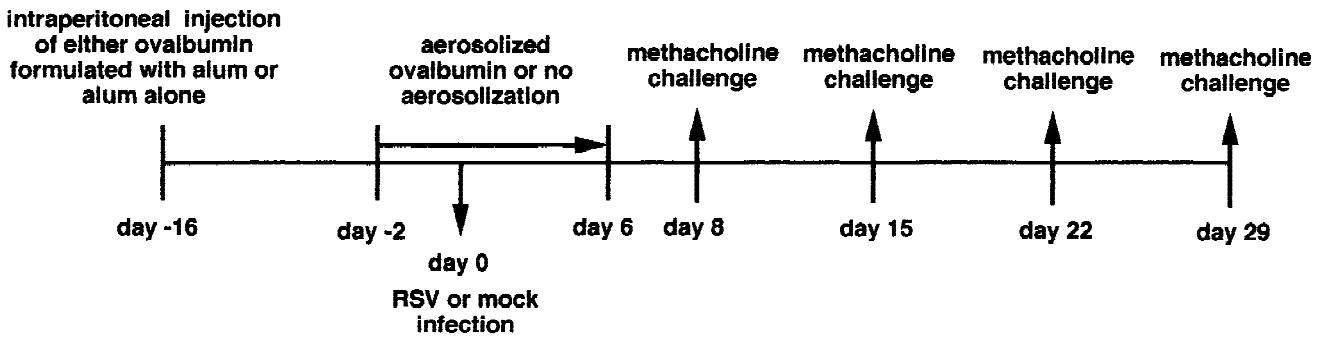

Fig. 1. Time line of experimental protocol.

position to aeroallergen sensitization (type I hypersensitivity) during the first years of life [Welliver and Duffy, 1993; Sigurs et al., 1995]. Thus, RSV bronchiolitis may be a risk factor for the development of both asthma and atopy. In mice, RSV infection increases the rate and amount of IgE production in mice subsequently exposed to aerosolized antigen [Leibovitz et al., 1988], and has been reported to increase airway responsiveness to methacholine [Schwarze et al., 1997]. In a guinea pig model, prior allergen sensitization potentiates airway reactivity induced by acute RSV bronchiolitis [Robinson et al., 1997].

Alternatively, asthma and atopy may be a risk factor for severe RSV-induced disease. In a bovine model of allergic inflammation, prior aeroallergen sensitization heightened the illness caused by subsequent RSV infection [Gershwin et al., 1990]. Calves sensitized to an aerosolized allergen and subsequently infected with RSV had increased RSV-specific IgE, worsened lung pathology, and heightened clinical disease on reexposure to the allergen compared with calves that were either allergen sensitized or RSV infected alone. The immunologic and physiologic mechanisms linking RSV with bronchospasm and allergic disease are undefined.

To explore the mechanisms by which airway inflammation caused by the interaction of virus-induced and allergic immune responses results in AHR, we developed a model combining airway allergen sensitization and RSV infection in mice. Ovalbumin sensitization alone is known to increase AHR and promote tissue and bronchoalveolar lavage (BAL) eosinophilia [Mehlhop et al., 1997], but the kinetics of the response has not been reported. We defined the time course of AHR in mice infected with RSV alone, sensitized with ova alone, and mice with combined ova-induced and RSV-induced inflammation. RSV infection significantly prolonged AHR in ova-sensitized mice. This finding suggests that virus-induced immune responses and allergic airway inflammation interact to produce altered responses to both virus and allergen with a significant effect on lung physiology. Development of this combined model of RSV-induced and allergic inflammation in an immmunologically tractable system provides the basis for future studies that will define the molecular mechanisms involved in regulating immune responses and promoting AHR in this setting.

METHODS

Mice

Pathogen-free 8-week-old female BALB/c mice were purchased from Harlan, Inc. (St. Louis, MO). They were shipped in filtered crates and housed in a HEPA-filtered Duo-flo laminar flow unit. Cages, bedding, food, and water were sterilized prior to use. Room temperature was maintained at 27°C and a 12-hr-on, 12-hr-off light cycle was provided. In caring for animals, the investigators adhered to the Guide for the Care and Use of Laboratory Animals prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, revised 1985).

Cells and Virus

HEp-2 cells were maintained in Eagle's minimal essential media (EMEM) supplemented with glutamine, amphotericin, gentamicin, penicillin G, and 10% fetal bovine serum (10% EMEM). The A2 strain of RSV was provided by Dr. Robert Chanock, National Institutes of Health. Master stocks and working stocks of RSV were prepared as described previously [Graham et al., 1988].

Allergen Sensitization Protocol

Mice received an intraperitoneal injection of 0.1 ml (10μg) of ovalbumin (chicken OVA, grade V, Sigma Chemical Co., St. Louis, MO) complexed with 20 mg of $Al(OH)_3$ on day −16 (Fig. 1). On days −2 through 6, the mice were placed in an acrylic box and exposed to aerosols of 1% ovalbumin diluted in sterile phosphate-buffered saline (PBS) using a jet nebulizer (Pari II, Pari Industries, Richmond, Virginia) for 40 min each day. Opposite the aerosol orifice was a small exhaust orifice connected to vacuum suction to ensure continuous air flow. Age-matched control animals received a mock sensitization with intraperitoneal $Al(OH)_3$ on day −16.

Mouse Infection

On day 0, the mice were infected with RSV or mock infected (culture media alone) intranasally as described previously [Graham et al., 1988]. Briefly, the mice were anesthetized with intramuscular ketamine 40 μg/g and xylazine 6 μg/g. When held upright with

the neck fully extended, the mice readily inhaled a 100-μl inoculum of undiluted stock virus (10^7 plaque forming units of RSV A) placed over their nostrils with a micropipette. RSV infection with this procedure causes bronchiolitis [Graham et al., 1988]. Infection was confirmed in a subset of mice by plaque assay in HEp-2 cells as described previously [Graham et al., 1988].

Study Protocol

The study protocol is outlined in Figure 1. The mice were divided into four groups. Group 1 was ovalbumin-sensitized and mock-infected (OVA). Group 2 was ovalbumin-sensitized and RSV-infected (OVA/RSV). Group 3 was mock-sensitized and RSV-infected (RSV). Group 4 was mock-sensitized and mock-infected (MOCK). Four mice in each group were sacrificed on day 4 of infection for plaque assay, confirming infection in the mice that underwent intranasal RSV administration. Six to 10 mice from each group underwent methacholine challenges on days 8, 15, 22, or 29 (1, 2, 3, or 4 weeks following RSV infection).

Methacholine Challenge

Mice were anesthetized with intraperitoneal injections of pentobarbital sodium (70 mg/kg) and a tracheostomy tube was placed. The internal jugular vein was cannulated and a microsyringe was attached to the intravenous tubing for methacholine administration. The mice were then placed in a whole body plethysmography chamber and ventilated mechanically [Chen et al., 1994]. Transpulmonary pressure was measured as the airway opening pressure referenced to pressure within the chamber. A four-way connector was attached to the tracheostomy tube. One port of the four-way connector was attached to the inspiratory side of a ventilator (Harvard rodent ventilator, model 683, South Natick, MA), while another port was connected to the ventilator expiratory side port. Mice were ventilated at a rate of 150 breaths per minute with a tidal volume of 5–6 ml/kg and a positive end expiratory pressure of 2–4 cm H_20. Lung volume changes were measured by detecting pressure changes in the plethysmographic chamber. Flow was measured by electronic differentiation of the volume signal. Pressure, flow, and volume changes were recorded (Validyne, model MC 1-3-871). Pulmonary resistance was calculated using a proprietary software program (LabVIEW Graphical Programming for Instruments, National Instruments, Version 3.1). Acetyl-β-methacholine (Sigma) was dissolved in normal saline and administered intravenously at a starting dose of 5 μg/kg. The average volume per methacholine dose was approximately 35 μl. Three-fold increasing concentrations of methacholine were administered at 5-min intervals and only after transpulmonary pressure and tidal volume returned to baseline. Pulmonary variables were recorded for at least 10 breaths during the peak response, approximately 30 sec after each intravenous methacholine dose. Methacholine dose response curves were obtained by calculating the mean ± standard error for individual animals at each methacholine dose.

BAL

Following lung resistance measurements, the animals were given a lethal injection of pentobarbital. BAL was then performed by instilling 600 μl of 5% bovine serum albumin diluted in normal saline through the tracheostomy tube and then withdrawing the fluid with gentle suction via the syringe. The typical BAL fluid return was 300–400 μl. White blood cells were counted on a hemocytometer. Cytologic examination was performed on cytospin preparations (Shandon Southern Instruments, Sewickly, PA). Cytospin slides were fixed and stained using Diff Quik (American Scientific Products, McGaw Park, IL). Differential counts were based on counts of 100 cells using standard morphologic criteria to classify the cells as either eosinophils, lymphocytes, or other mononuclear leukocytes (alveolar macrophages and monocytes).

Protocol for Examining Lung Sections

The lungs were inflated via the tracheostomy tube with 10% phosphate-buffered formalin. The left lung was then removed and stored in 10% phosphate-buffered formalin, paraffin-embedded, cut in 6-μm sections, mounted, and stained with hematoxylin and eosin for routine histology, periodic acid-Schiff (PAS) to assess mucus, and Luna stain to specifically evaluate eosinophils [Luna, 1979]. Slides were examined in their entirety at 10× magnification by one observer (J.E.J.) in a blinded fashion. The following compartments of the lung were assessed: alveolar spaces, airways at all levels, interstitium, and vessels (both arteries and veins). Inflammatory infiltrates were evaluated for location, severity, and composition (cell types: small mononuclear cells, transformed lymphocytes, histiocytes, neutrophils, eosinophils). Airway types were defined as follows: alveolar duct = walls consisting entirely of alveoli; respiratory bronchiole = occasional mural alveoli, low cuboidal epithelium; terminal bronchiole = low cuboidal epithelium, intact walls; bronchiole = respiratory epithelium, no cartilage; bronchus = respiratory epithelium and cartilage. Vessels were defined as arteries if they traveled with airways and had a thick media, and veins if they were thin-walled and unaccompanied by airways. Cell types were defined as follows: lymphocytes = small mononuclear cells; transformed cells = larger mononuclear cells with moderate basophilic cytoplasm and nuclear clearing with large, coarse nucleoli; histiocytes = the largest cells, with generous eosinophilic cytoplasm and small nucleoli; eosinophils = cells with segmented nuclei and bright red refractile granules; neutrophils = cells with segmented nuclei without refractile red granules. The perivascular 'cuff thickness' was assessed semiquantitatively as a measure of severity of inflammation and was evaluated at the point of minimal diameter of the structure. The degrees of inflammation were graded as follows: 0 = no infiltrate; 1+ = most

vessels have an infiltrate up to 4 cells thick; 2+ = most vessels have an infiltrate 5–7 cells thick; 3+ = most vessels have an infiltrate greater than 7 cells thick. Interstitial alveolar cellularity was graded as follows: 0 = no infiltrate; 1+ = minimal increased cellularity without widening of septa; 2+ = obvious increased cellularity with widening of septa; and 3+ = markedly increased cellularity with thickened septa; this score also includes blood or edema fluid in the tissue space.

Statistical Analysis

Results are expressed as mean ± standard error of the mean (SEM). Cellular composition of the BAL fluid and lung histopathologic results were compared with a Mann–Whitney *U* test for unpaired data. Dose–response curves to methacholine were compared by repeated measures analysis of variance (ANOVA) with Fisher's PLSD performed as a post hoc analysis. Differences were considered to be significant if $P < .05$.

RESULTS

Time Course of Methacholine-Induced Airway Responsiveness

Airway responsiveness to intravenous methacholine was measured in mechanically ventilated mice in a similar fashion to that used by many other investigators [Martin et al., 1988; Chen et al., 1994; Gavett et al., 1995; Henderson et al., 1997]. There was no significant difference in baseline airway resistance between the OVA and OVA/RSV groups (Fig. 2). While the baseline resistance of the RSV group was significantly greater than that of the MOCK group ($P < .05$) on days 8 and 15, RSV infection alone did not cause significant AHR. On day 8 (1 week after RSV or mock infection), the OVA mice and OVA/RSV groups had significantly greater airway responsiveness to methacholine than the RSV or MOCK groups.

By day 15 (2 weeks after RSV or mock infection), AHR in the OVA group was not significantly greater than the RSV or MOCK groups. In striking contrast, the OVA/RSV group had significant AHR at the 2-week time point ($P < .01$) compared with any of the other three groups.

By day 22 (3 weeks after RSV challenge), the AHR of the OVA/RSV group had decreased to the level of the other groups. On day 29, there was no difference in AHR between the four groups (data not shown).

Cellular Composition of the Lung Inflammatory Responses

On day 8, eosinophils constituted the major inflammatory cell in BAL fluid from either the OVA or OVA/RSV groups (data not shown). The number of eosinophils in these two groups was significantly greater than for either the RSV or the MOCK groups, in which macrophages composed the majority of BAL cells. There was no difference in the number of lymphocytes between the OVA, OVA/RSV, and the RSV groups on day 8.

On day 15, the number of eosinophils in the BAL fluid of the OVA/RSV and OVA groups were not different (Fig. 3). However, the number of lymphocytes in the OVA/RSV and RSV groups were significantly greater than in the OVA and MOCK groups ($P < .05$). The number of macrophages were not statistically different in the OVA/RSV and RSV groups compared with the OVA and MOCK groups.

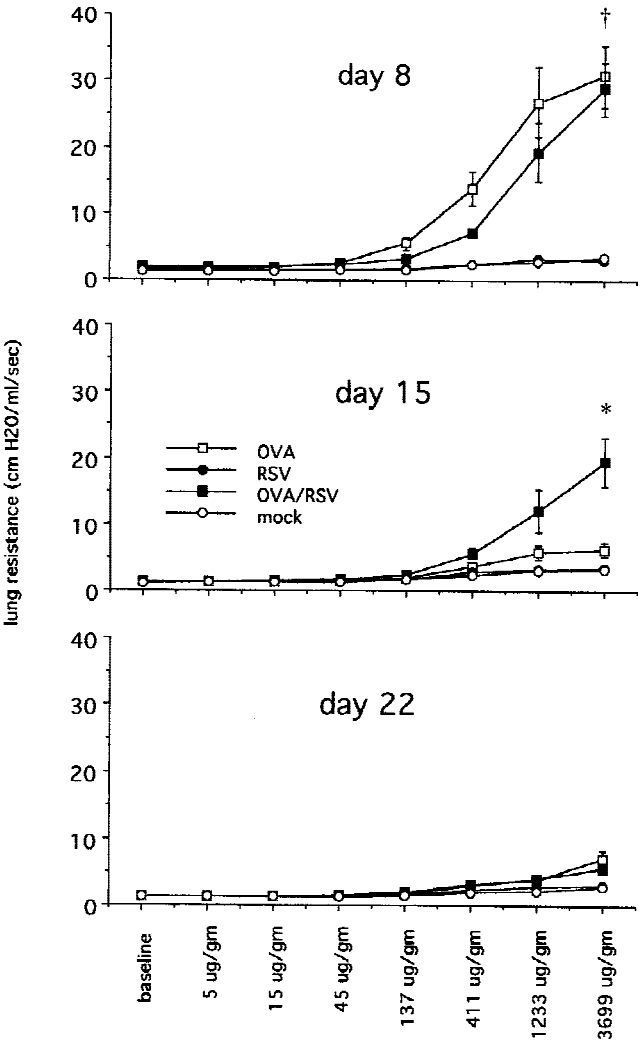

Fig. 2. Results of methacholine challenge performed on days 8, 15, and 22 (1, 2, and 3 weeks after either respiratory syncytial virus [RSV] or mock infection, respectively). Lung resistance is measured as cm H_2O/ml/sec. Data points represent the mean ± SEM from 6–10 mice. OVA/RSV, ▲; OVA, ○; RSV, ■; mock, □. †$P < .01$ OVA/RSV and OVA compared with other groups; *$P < .01$ OVA/RSV compared with other groups.

Plaque Assays and Weight Changes

Plaque assays were performed on all four groups of mice on the fourth day after either RSV or mock infection. The OVA and MOCK groups had no evidence of viral plaque formation. The number of plaques in the RSV and OVA/RSV group were similar (mean 6.5 $\log_{10}$ plaque forming units/gram lung) to those that we have previously seen with the same inoculum [Graham et al., 1988]. There was no difference in the number of

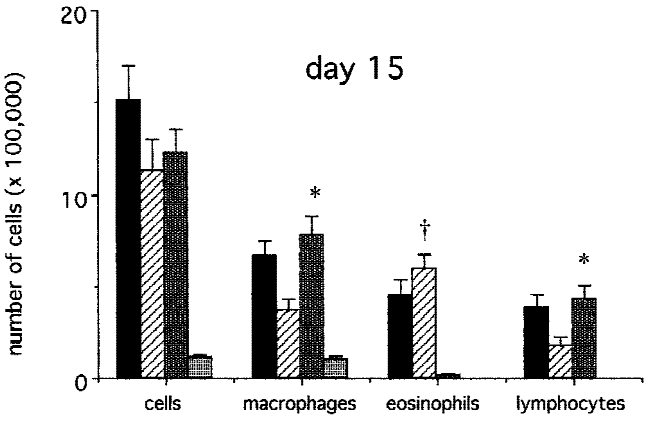

Fig. 3. Composition of cell counts from bronchoalveolar lavage (BAL) fluid on day 15. OVA/RSV, ■; OVA, ▨; RSV, ■; mock, ▦;. *P < .05 OVA/RSV and RSV compared with other groups; †P < .05 OVA/RSV and OVA compared with other groups.

plaques between the RSV and OVA/RSV groups in this experiment (data not shown). There was no statistically significant difference in the weights between the groups at baseline; however, at day 6 after infection, both the RSV and OVA/RSV mice had significantly decreased weights compared with the MOCK and OVA groups (P < .05). At day 8 after infection, the OVA/RSV mice had returned back to their baseline weight, while the RSV mice still had significantly decreased weight compared with the other three groups (P < .05). By day 10, the weight of the RSV mice had returned to the same level as the other three groups of mice.

Histologic Changes

The inflammatory characteristics caused by allergic sensitization and infection are summarized in Figure 4. The OVA/RSV mice had a greater degree of interstitial and alveolar inflammation than the OVA group at days 8, 15, and 22. At day 8, the RSV mice had the greatest degree of interstitial and alveolar inflammation compared with any of the groups of mice at this time period; however, at the later time points, the interstitial and alveolar inflammation was less than that of the OVA/RSV mice. The MOCK group had a slight degree of interstitial and alveolar inflammation at day 8 that was less than any other group and had no interstitial and alveolar inflammation at the later time points.

Peribronchiolar inflammation in the OVA/RSV and OVA mice was greater at days 8 and 15 compared with both the MOCK and the RSV groups (Fig. 4). At day 15, the peribronchial infiltrate in the OVA mice was composed primarily of eosinophils, whereas the infiltrate in the RSV mice consisted mainly of mononuclear cells. However, the peribronchial infiltrate in the OVA/RSV mice had an equal number of both eosinophils and mononuclear cells. The MOCK group of mice had no peribronchial inflammation at any time point.

The distribution and magnitude of mucus production in the airways was different between groups. In the MOCK group, PAS positivity was limited to large cartilaginous airways (bronchi), and was observed in an apical distribution in occasional cells. In the MOCK mice, bronchioles were consistently negative for magenta staining. In the RSV group, the number of PAS-positive cells in cartilaginous airways was moderately increased, and intensity of staining in those cells was

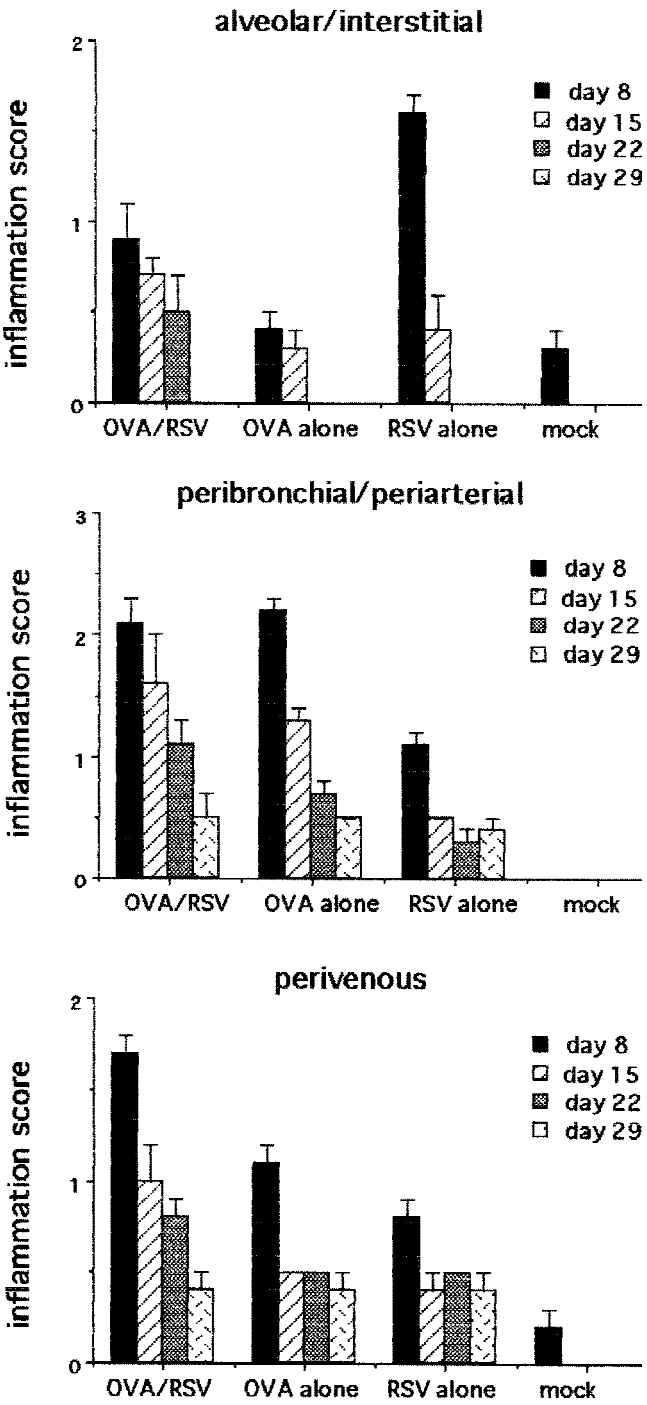

Fig. 4. Inflammation scores form the lung histopathology sections in the alveolar/interstitial, peribronchial/periarterial, and perivenous spaces. Day 8, day 15, day 22, and day 29 were 1, 2, 3, and 4 weeks after either respiratory syncytial virus (RSV) or mock infection, respectively. Error bars represent SEM.

increased compared with MOCK; bronchioles of all sizes were without PAS-positive cells, as in the MOCK mice. Ovalbumin sensitization, with or without RSV infection, was consistently associated with the appearance of numerous PAS-positive cells in the mid-sized bronchioles. Only in the OVA/RSV mice were both cartilaginous airways and bronchioles observed to have numerous PAS-positive cells. A single intrabronchiolar mucus pool was seen in one animal in the OVA group; these were present in four of five OVA/RSV mice and in this group the mucus pools were more numerous.

DISCUSSION

We have defined the time course of airway responsiveness in mice sensitized with ovalbumin alone, RSV-infected alone, or mice with combined ova-sensitization and RSV infection. RSV infection during ovalbumin sensitization substantially prolonged the period of AHR. Two weeks after RSV challenge, ovalbumin-sensitized mice had significant AHR compared with mice sensitized with ova alone. RSV infection alone did not cause significant AHR, suggesting that prior allergic inflammation altered the response to RSV. Neither illness nor magnitude of RSV replication correlated with AHR seen in our model 2 weeks after viral infection. The murine response to primary RSV infection is a 5–7-day period of weight loss and decreased activity, with peak illness occurring between days 8–10 of infection [Graham et al., 1988]. Illness associated with primary infection is self-limited with full recovery by day 12 [Graham et al., 1988]. On day 15, at the time of enhanced AHR in this study, mice had completely recovered from illness induced by RSV infection.

At day 15, the distribution of mucus-producing, PAS-positive cells was strikingly different between the four groups. The large airways of the RSV mice had an increase in PAS-positive cells compared with the MOCK group, while there was an increase in PAS-positive cells only in the smaller airways (mid sized bronchioles) of the OVA group compared with the MOCK mice. However, in the OVA/RSV mice, there was an increase in PAS-positive cells in both the larger cartilaginous and smaller airways. This alteration in distribution of mucus production was accompanied by increased intraluminal pools of mucus in the OVA/RSV mice compared with both the OVA and other two groups. Others have also suggested that RSV induces emptying of mucin from the airway cells in ovalbumin-sensitized mice [Blyth et al., 1997]. This increase in the distribution of PAS-positive cells and increased intraluminal mucus in the OVA/RSV mice could provide an explanation for the increased baseline lung resistance and AHR compared with the other three groups at this same time point, and suggests that RSV could cause the release of mucus secretagogues such as leukotriene C_4 (LTC_4), prostaglandin D_2 (PGD_2), or histamine. Interstitial inflammation could affect airway mechanics by producing soluble mediators that promote intraluminal mucus accumulation.

The numbers of eosinophils does not explain the difference in airway responsiveness to methacholine seen 2 weeks after RSV challenge in the OVA/RSV group compared with the OVA mice. Mice sensitized with ovalbumin alone had a greater percentage and a similar total number of eosinophils in BAL fluid compared with the OVA/RSV mice, but less airway responsiveness. Although the level of eosinophil activation has not yet been assessed, the total number of eosinophils in BAL fluid would suggest that the presence of eosinophils is associated with AHR, but eosinophils are not sufficient for induction of AHR by themselves. This hypothesis is consistent with a finding in BALB/c mice sensitized with ovalbumin alone, in which eosinophils alone are not sufficient to explain changes in AHR [Corry et al., 1996].

Although no significant difference was observed in the number of eosinophils in BAL fluid at day 15 between the OVA/RSV and OVA groups, there was a greater peribronchiolar lymphocytic inflammation at day 15 in the OVA/RSV group compared with either the OVA, RSV, or MOCK groups at the same time point. Neutrophils were not seen in the BAL fluid or in the lung tissue. We have therefore begun to explore mechanisms by which T lymphocytes or virally infected cells such as epithelial cells modulate AHR.

Using knock-out mice, others have shown that airway reactivity in ovalbumin-sensitized mice is not fully dependent on either IgE [Mehlhop et al., 1997], B lymphocytes [Hamelmann et al., 1997], or mast cells [Takeda et al., 1997]. This observation is consistent with T lymphocytes being an important constituent of allergen-induced airway hyperresponsiveness. Indeed, adoptive transfer of CD4+ T lymphocytes alone can result in airway responsiveness to allergen challenge in rats [Watanabe et al., 1997], and inhibition of T cell stimulation inhibits airway hyperresponsiveness in allergen-sensitized mice [Krinzman et al., 1996].

T lymphocytes are an integral component of the murine immune response to RSV. We have shown that both CD4+ and CD8+ cytotoxic T cells are important for terminating RSV replication in primary RSV infection in mice, and both T-cell subsets mediate illness in primary RSV infection [Graham et al., 1991]. The typical response to RSV infection in naive BALB/c mice includes a type 1 pattern of cytokine expression [Graham et al., 1993] and like most RSV-infected children, the clinical syndrome is self-limited and without sequelae. Severe RSV-induced disease occurs in a small subset of children or in mice that have been manipulated by prior antigen-sensitization or interference with normal cytokine expression. Therefore, we suggest that the T-cell response to RSV is altered by the milieu associated with the allergic inflammation produced by allergen sensitization. The RSV-specific T-cell response, in turn, potentiates the allergen-induced inflammation, prolonging the release of cytokines and other mediators that are responsible for AHR.

Our results differ from two other reports that studied the effect of RSV infection on AHR. Schwarze et al.

found increased AHR in BALB/c mice that were challenged with 50μl containing 10^5 pfu of RSV when aerosolized methacholine challenge was performed on day 6 in a free range (Buxco) apparatus [Schwarze et al., 1997]. The 10^5 pfu inoculum in our model does not result in consistent histopathologic changes in the lung [Graham et al., 1988]. We performed measurements of AHR in mechanically ventilated mice through a tracheotomy tube to negate the effect of nasal obstruction from intranasal virus administration on airway reactivity measurements. Mice challenged with 10^4, 10^5, 10^6, or 10^7 pfu have no change in AHR compared with mock-infected mice on day 6 measured by our system (data not shown). Because we saw no increase in AHR to RSV infection, nasal mucosal reactivity to methacholine may possibly account for the some of the heightened responsiveness seen in the report by Schwarze and colleagues. Robinson and colleagues reported that primary RSV infection caused AHR to methacholine in guinea pigs [Robinson et al., 1997]. RSV infection in guinea pigs produces more desquamation of respiratory epithelium than in BALB/c mice, and may thereby lead to AHR through obstruction of small bronchioles.

In summary, RSV infection during ovalbumin sensitization prolonged the period of AHR, which correlated with increased numbers of lung lymphocytes and airway mucus production. Developing the combined model of allergen sensitization and RSV infection in an immunologically tractable system provides the basis for exploring the molecular and cellular mechanisms responsible for methacholine-induced airway hyperresponsiveness.

ACKNOWLEDGMENTS

We thank Dr. Tina Hartert for her assistance in statistical analysis. This project was supported by the Vanderbilt University Grant Liddle Fellowship Award.

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